Equivalence Studies for Complex Active Ingredients and Dosage Forms

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Lokesh Bhattycharyya,[1] Roger Dabbah,[1] Walter Hauck,[2] Eric Sheinin,[1] Lynn Yeoman,[3] and Roger Williams[1]

[1]United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790
[2]Biostatistics Section, Thomas Jefferson University, Philadelphia, PA 19107
[3]Baylor College of Medicine, Houston, TX 77030

ABSTRACT

This article examines the United States Pharmacopeia (USP) and its role in assessing the equivalence and inequivalence of biological and biotechnological drug substances and products—a role USP has played since its founding in 1820. A public monograph in the *United States Pharmacopeia–National Formulary* helps practitioners and other interested parties understand how an article's strength, quality, and purity should be controlled. Such a monograph is a standard to which all manufactured ingredients and products should conform, and it is a starting point for subsequent-entry manufacturers, recognizing that substantial additional one-time characterization studies may be needed to document equivalence. Review of these studies is the province of the regulatory agency, but compendial tests can provide clarity and guidance in the process.

KEYWORDS: US Pharmacopeia, biological or biotechnological drug, equivalence, generic biologics, complex active ingredient

INTRODUCTION

USP (an acronym for the United States Pharmacopeial Convention, Inc., which was incorporated in 1900 in the District of Columbia) was formed in 1820 by practitioners who wished to standardize the recipes (process standards) used to prepare pharmaceuticals and give them unique, clear, and useful names. With the rise in modern pharmaceutical manufacturing, this role has changed so that USP now provides product standards for therapeutic ingredients and dosage forms to assure their strength, quality, and purity. These ingredients and dosage forms are termed articles, as in articles of commerce, both in the *United States Pharmacopeia* and the *National Formulary (USP–NF)* and also in the United States Federal Food, Drug, and Cosmetic Act (FFDCA). Therapeutic articles include biologics, chemically synthesized drugs, excipients, dietary supplements, and some devices. Standards are available for more than 4000 ingredient and dosage form monographs in *USP–NF*. These standards include the article's definition, description, brief packaging, storage, and labeling statements, and a specification (ie, tests, procedures, and acceptance criteria). A monograph is intended to be unambiguous so that any individual with the requisite training and equipment can successfully conduct the required tests. If an article meets the stipulations of the monograph when tested, then its identity, under its name, is established. *USP–NF* still contains a small number of recipes for use by compounding practitioners, reflective of the intent of the early Pharmacopeia.

As a nonprofit 501 (c)(3) corporation, USP differs from most pharmacopeias of the world insofar as the latter typically function in close association with one or more governments and may be governmental or quasi-governmental bodies.

The governing bodies of USP (Convention and Board of Trustees) as well as its standards-setting body (Council of Experts) are composed entirely of volunteers. Their mission is to promote

… the public health by developing and disseminating quality standards and information for medicines, health care delivery, and related products and practices. Our standards and information help patients and practitioners maintain and improve health.[1]

At the direction of its Board of Trustees, USP publishes *USP–NF* annually with 2 *Supplements*. These texts are continuously revised to account for new ingredients and products and for advances in analytical procedures. *USP* and *NF* are named as official compendia in the FFDCA and are referenced in other laws and regulations, not only of the United States but of other countries as well.

USP is guided by resolutions adopted at the USP Convention, which meets every 5 years. Delegates to the March 2000 Convention endorsed 19 resolutions, one of which (Resolution 2) encouraged USP to perform the following:

Explore the feasibility and advisability of developing guidance on principles and approaches to assure equivalence of complex active ingredients (including botanicals and dietary supplements) recognizing the special issues associated with agents of biologic/biotechnological origin, including their regulatory control.[2]

With encouragement and oversight from the Board of Trustees, USP formed an Expert Panel to consider this resolution.

Corresponding Author: Roger Williams, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790. Tel: (301) 816-8300; Fax: (301) 816-8299; E-mail: rlw@usp.org

In November 2003, USP also convened a conference titled *Biological and Biotechnological Drug Substances and Products*, in which Expert Panel presentations were discussed publicly.[3] This report represents a synthesis of the various perspectives and presentations and is designed to be of use to USP volunteer bodies, practitioners, policy makers, manufacturers, other compendia, regulatory agencies, and the public at large. A general theme of this report is that the science and technical issues can be readily understood by all parties, using appropriate risk assessment and management, and the scientific method. A history of USP's involvement in the general topic of complex active ingredients appears in Appendix 1 to this article.

DEFINITIONS

When a practitioner or consumer uses a drug, generally it is in a dosage form that contains a drug substance and one or more excipients. Drug substances may be categorized by type or source. By type, drug substances may be complex or noncomplex; by source, complex drug substances may be from natural sources (eg, plants and/or animals, including humans) or produced by recombinant DNA (rDNA) techniques—hence the terms biological and biotechnological drug substances and products. Below are a series of definitions of a biological substance.

World Health Organization

In the context of biological standardization, the World Health Organization (WHO) has defined a biological substance as "a substance which cannot be completely characterized by physicochemical means alone and which therefore requires the use of some form of bioassay."[4] These assays involve comparison of the response of the test substance with that of a reference material. Since the 1920s, WHO has supplied international biological reference materials for such procedures.

United States Federal Food, Drug, and Cosmetic Act

Under the Act, biological and biotechnological medicinal products are considered drugs, and the term *drug* is defined as follows[5]:

1. articles recognized in the official *United States Pharmacopoeia,* official *Homeopathic Pharmacopoeia* of the United States, or official *National Formulary,* or any supplement to any of them; or
2. articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in humans or other animals; or
3. articles (other than food) intended to affect the structure or any function of the body of humans or other animals; or
4. articles intended for use as a component of any article specified in clause (A), (B), or (C).

United States Public Health Service Act

A biological product subject to licensure under the United States Public Health Service Act (PHSA) is any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, applicable to the prevention, treatment, or cure of diseases or injuries to humans.[6] Biological products include, but are not limited to, bacterial and viral vaccines, human blood and plasma and their derivatives, and certain products produced by biotechnology, such as interferons and erythropoietins.

United Kingdom

The United Kingdom Biological Standards Act states that biologics are substances "whose purity or potency cannot be adequately tested by chemical or physical means."[7]

Other definitions of biologicals include the following:

- Products of living organisms used in the prevention or treatment of disease.[8]
- A classification of products derived from living sources, such as humans, animals, bacteria, and viruses. Vaccines, immune globulin, and antitoxin are biologics.[9]

From the above, it is clear that consensus does not yet exist for a general definition of a biological or for ways to distinguish between complex and noncomplex active ingredients. However, as a general approach that forms the basis for the subsequent discussion (and recognizing many areas of overall agreement), a complex active ingredient may be viewed simply as one that (1) comes from living organisms and/or (2) cannot be fully characterized by physical and/or chemical means. (During the course of the 2000-2005 cycle, USP's method of dealing with Resolution 2 became more focused, and botanicals and dietary supplements were removed from consideration.) In this article, the general term *complex active ingredient/product* is used interchangeably with the general term *biological or biotechnological drug substance and product*.

EQUIVALENCE CONCEPTS

Conditions When Similarity Questions Arise

Issues of similarity or lack thereof have been a theme of USP since its founding in 1820. In fact, the preface to the first edition of USP in 1820 stated:

It is the object of a Pharmacopoeia to select from among substances which possess medicinal power, those, the utility

of which is most fully established and best understood; and to form from them preparations and compositions, in which their powers may be exerted to the greatest advantage. It should likewise distinguish those articles by convenient and definite names such as my prevent trouble or uncertainty in the intercourse of physicians and apothecaries.[10]

Irrespective of proprietary naming considerations, therapeutic products that are therapeutically equivalent (ie, pharmaceutically equivalent and bioequivalent) should bear the same names; therapeutic products that are not should bear different names. Issues of equivalence arise continuously at various points in the life cycle of a manufactured drug substance and product. At least 3 can be clearly identified. The first is batch-to-batch equivalence when no change in method of manufacture or ingredients has occurred. This type of equivalence may be referred to as batch-to-batch consistency. The second is when a manufacturer makes one or more specified changes to ingredients or method of manufacture. This type of equivalence is sometimes termed comparability. The third is when one manufacturer attempts to create a duplicate of another manufacturer's product, using different procedures, and at times different specifications. For a biological and biotechnological product, this type of equivalence is sometimes referred to as generic biologic, biogeneric, or follow-on biologic. The different terms used to express equivalence concepts—similar, same, identical, essentially similar, comparable, interchangeable, therapeutically equivalent, pharmaceutically equivalent, bioequivalent, follow-on biologic, and biogeneric—have varying scientific and legal meanings.[11,12] Depending on the degree of change, or, in the case of batch-to-batch consistency, when there is no deliberate change at all, the type and amount of data to demonstrate equivalence varies. This report focuses on general concepts and approaches to assess equivalence, irrespective of the specific setting in which the question arises. A risk management, assessment, and communications approach will help determine the number and types of tests that will be needed according to a specified degree of change. This consideration is beyond the scope of this report but has been taken up by the United States Congress in the 1997 Food and Drug Administration Modernization Act (FDAMA),[13] in an International Conference on Harmonization (ICH) guideline,[14] in various FDA guidances,[15,16] and in other regulatory documents.[17,18]

Equivalence Approaches: Hypothesis Testing

The scientific method begins with observation, which leads to a hypothesis (deductive reasoning). The hypothesis suggests an experimental study (inductive reasoning) that can refute or confirm but can never unequivocally establish the hypothesis. Hypothesis testing usually begins with an assumption of no difference (the null hypothesis). If the experiment allows rejection of the null hypothesis, a difference may be concluded (the alternative hypothesis). In modern drug development, this approach is frequently used (eg, to test an active treatment compared with placebo). Equivalence testing is the reverse, where the null hypothesis is inequivalence and the alternative hypothesis is equivalence.[12] If the experiment allows rejection of the null hypothesis, equivalence is concluded. An equivalence approach uses a criterion, which forms the basis for the comparison, and equivalence limits (acceptance criteria), which are predetermined boundaries of *in*equivalence. These are nonstatistical judgments made by regulators, pharmacopeias, manufacturers, and others. Statistical tests are used to determine whether comparative data in an experimental population allow rejection of the null hypothesis of inequivalence and acceptance of the alternative hypothesis of equivalence. A standard approach[19] uses a confidence interval (eg, 90%) to test equivalence; the observed interval (the 90% confidence interval for the mean test and reference difference in the experimental population) must fall completely within predetermined acceptance criteria (eg, 80%-125%). Additional approaches that better account for variance also have been considered.[20]

Resolution 2

The 2000 USP Convention's Resolution 2 refers to complex active ingredients. These now include a broad range of ingredients and their corresponding dosage forms, including proteins, blood and blood products, vaccines, and cell- and gene-therapy products. The categories and classes within categories are expected to increase in the coming years, as therapeutic products from the molecular biology revolution increasingly become available. In considering Resolution 2, the Expert Panel agreed to focus on protein-based complex active ingredients and their corresponding dosage forms, given that these now form the bulk of biological and biotechnological therapeutic products. They can be further classified as peptides, nonglycosylated proteins, glycosylated proteins, and monoclonal antibodies (Figure 1). Although Resolution 2 encouraged USP to also consider botanicals and dietary supplements, the Expert Panel did not include these types of ingredients and products in their deliberations. Many of the principles and approaches discussed for protein and other complex active ingredient drug products are, however, applicable to botanicals and dietary supplements.

Expert Panel Presentations and Discussion

Equivalence experiments can rely on a broad spectrum of marketplace surveillance, clinical benefit, pharmacodynamic, pharmacokinetic, and nonclinical (animal) studies as well as physicochemical procedures. Depending on the

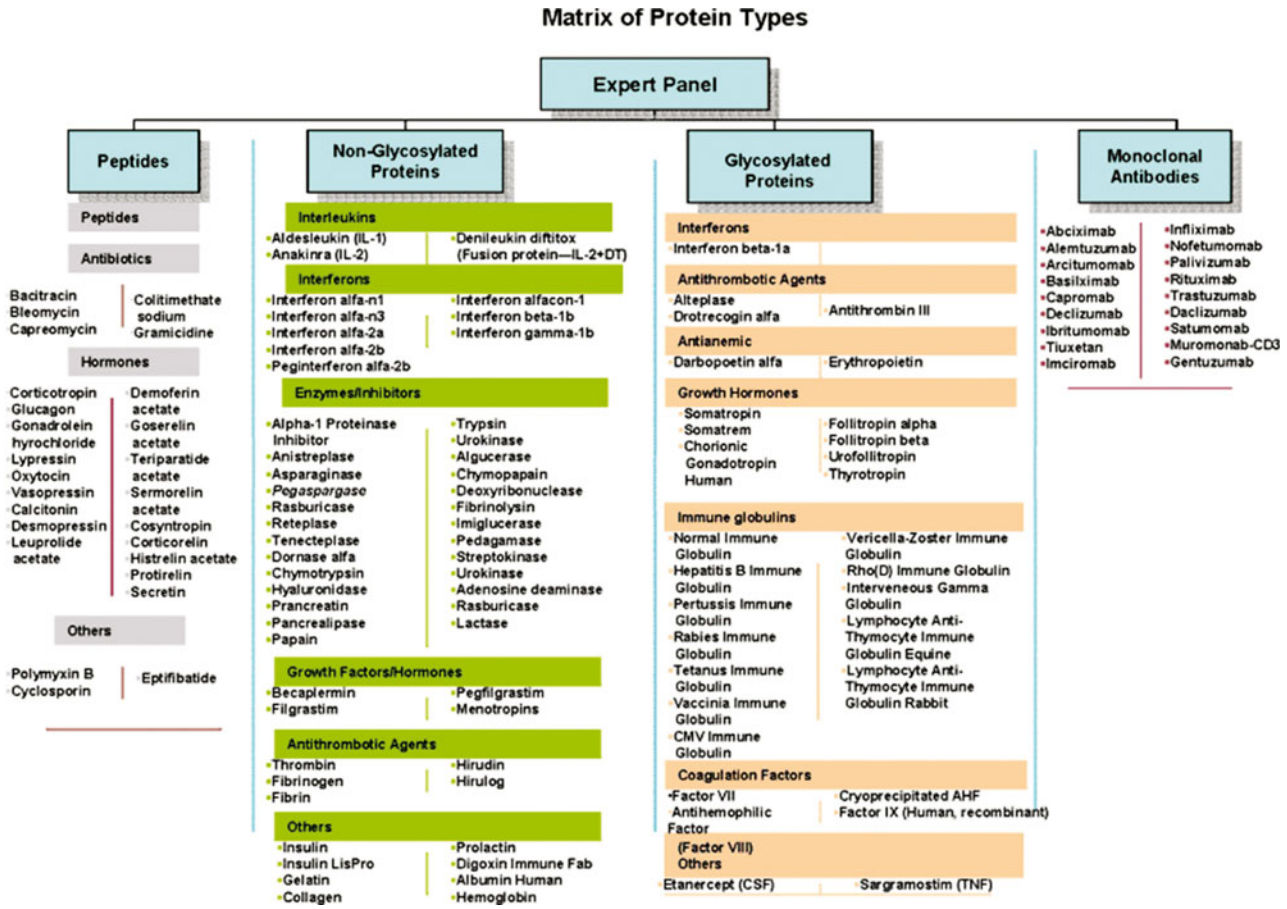

Figure 1. Matrix of protein types. (Reproduced with permission from USP.)

procedures called for in an equivalence experiment, scientists must decide which measurements (end points) should be used for making comparisons. For clinical studies, these may be positive (eg, blood pressure lowering, rise in blood count, time to survival) or negative (eg, headache, fatigue) therapeutic outcomes. Special measurements, sometimes drawn from appropriate models, may be used for pharmacodynamic and pharmacokinetic studies. Physicochemical measurements include a broad array of approaches based on rapid advances in analytical procedures. Procedures can be used primarily for characterization or can become part of a specification.

The selection of procedures to demonstrate equivalence will depend on the nature of the products, the private and/or public historical data already available, and the regulatory requirements. In process controls and end product specifications should be suitable to document batch-to-batch consistency. For intra- or intermanufacturer changes, a broad array of procedures may be required to demonstrate equivalence. The conclusions of presentations from the Expert Panel at a 1-day workshop at the USP Scientific Conference of November 2003 are briefly summarized below with grateful acknowledgments[21]; however, any opinions expressed here are solely those of the authors. These presentations were provided conceptually as a matrix, where the types of protein products were considered in terms of procedures that might be used to assess equivalence (Tables 1, 2, 3, 4, and 5).

Matrix of Peptides and Proteins

Peptides

Peptides consist of generally between 10 and 40 amino acid residues. They are used in foods and in both human and animal health products. In human health products, they function as antibiotics, growth promoters, immunomodulators (both stimulants and suppressants), and agents to treat diabetes, pain, hypertension, and infertility. Examples include oxytocin, desmopressin, glucagons, secretin, calcitonins, leuprolide, somatostatin, and cyclosporine. Three major synthetic strategies for a peptide are (1) chemical (both solid-phase and solution-phase); (2) biochemical (eg, fermentation); and (3) rDNA technology. *Chemical synthesis* is by far the most common approach, with approximately half of manufacturers using solution-phase methods and half using solid-phase methods. Chemical synthesis must be controlled carefully to ensure completion of deprotection and coupling reactions, stability of side-chain blocking groups and peptide-resin bonding, and removal of

Table 1. Analytical Procedures That Can Be Used to Assess Equivalence of Ingredients and Products of Biotechnological Origin*

Analytical Technology	Identity	Qty	Purity	Structure	Heterogeneity	Activity	Stability
Amino acid analysis	X	X	X				
Amino acid sequencing (C-, N-terminus)	X			X			
Biochemical/colorimetry (eg, S-S bonds)	X	X		X		X	X
Surface plasmon resonance	X					X	X
Capillary zone electrophoresis	X	X	X		X		X
Carbohydrate mapping	X			X	X		
Cell-based assays	X		X			X	X
FACS						X	X
HPLC (IEC, SEC, RP)	X	X	X	X	X		X
Immunoassays ELISA	X	X	X	X		X	X
Isoelectric focusing	X		X		X		X
LC-MS, CE-MS	X		X	X	X		X
Mass spectrometry	X		X	X	X		X
PCR, RTPCR, QPCR	X	X	X				
Microbiology (endotoxin, bioburden)			X				
Nuclear magnetic resonance	X	X		X			
Peptide mapping	X			X	X		X
Residual DNA			X				
SDS-PAGE (reduced and nonreduced)	X	X	X		X		X
Spectroscopy (UV, CD, IR, fluorescence)	X	X		X			
Ultracentrifugation (analytical)				X	X		X
Western blot	X		X	X			X

*FACS indicates fluorescence-activated cell sorter; HPLC, high-performance liquid chromatography; IEC, ion-exchange chromatography; SEC, size-exclusion chromatography; RP, reversed phase; ELISA, enzyme-linked immunosorbent assay; LC-MS, liquid chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; PCR, polymerase chain reaction; RTPCR, reverse transcription PCR; QPCR, quantitative PCR; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; CD, circular dichroism; and IR, infrared.

side-chain blocking groups. Because synthesis occurs through a series of steps, yield progressively decreases, with the increasing probability of truncated and internal deletion sequences. Common degradation products arising during synthesis include (1) asparagine deamidation, which generates aspartic acid and isoaspartic acid residues; (2) succinimide formation from aspartic acid, which is a precursor of isoaspartic acid; and (3) pyroglutamide formation from N-terminal glutaminyl peptides. Racemization is also a problem, which can be controlled and analyzed using various approaches. *Biochemical synthesis* may result in production of several species (eg, bacitracin), which can be acceptable in certain clinical settings. Stereospecificity is a major advantage of biosynthesis. *rDNA synthesis* is generally used for larger peptides such as growth hormone and insulin. rDNA synthesis of peptides can rely on *Escherichia coli*, yeast, and mammalian host cells. This approach frequently results in a mixture of closely related species. Peptides produced in the host cell may not be stable, requiring fusion to a larger protein (eg, beta-galactosidase) and subsequent cleavage. Translational fidelity with both mistranslation (occasional error in amino acid incorporation) and misincorporation (incorporation of wrong amino acid) can be a problem. Undesired posttranslational modifications may also occur. Various approaches to minimize these changes can be selected. Despite the small number of amino acid residues, peptides may have significant structural characteristics, which presumably can affect clinical performance.

In contrast, because of the relatively small number of amino acid residues, peptides can be more thoroughly characterized than proteins. Purity of peptides may be determined by a variety of methods. Although physicochemical characterization and purity analyses are more straightforward than for proteins, these methods may still not be sufficient to predict biologic toxicity and immunogenicity.

Nonglycosylated Proteins

Many of the issues associated with peptides are also common to nonglycosylated proteins, and additional issues arise as well. Variants of the desired molecule can be produced during synthesis, by chemical or physical reaction with manufacturing materials or components, or through degradation. For this reason, nonglycosylated proteins arising from rDNA synthesis tend to be heterogeneous. Additional complexity arises because of complex interactions of

Table 2. Physicochemical Tests for Peptides Analysis*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
pH	pH	P	X	X	<791>
Identification of active ingredients	SDS-PAGE	I/P	X	X	<726> <1047>
	Western blot	I/P	X	X	
	CE (free solution/gel filled)	I/P	X	X	<727> <1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	MALDI-TOF	I/P	X	X	
	Mass spectrometry (LC-MS/CE-MS)	I/P	X	X	<736>
	Spectrophotometry	I/P		X	<197>
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X	X	
	Ligand binding activity (see below)	I/P	X	X	
Purity	SDS-PAGE	I/P	X	X	<726> <1047>
	CE (free solution/gel filled)	I/P	X	X	<727> <1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	Mass Spectrometry (LC-MS/CE-MS)	I/P	X	X	<736>
	MALDI-TOF	I	X	X	<736>
	NMR	I	X	X	<761>
	Spectrophotometry	I/P		X	<851>
Dissociation/truncation/deletion	SDS-PAGE	I	X	X	<1047>
	SDS-CE (free solution/gel filled)	I	X	X	<1047>
	CIEF	I	X		<1047>
	MALDI-TOF/MS	I	X	X	<736>
	LC-MS/CE-MS	I	X	X	<736>
	HPLC size exclusion chromatography (HP-SEC)	I	X	X	<621>
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X	X	
Oxidation	RP-HPLC	I/P	X	X	<621>
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X		
	C-terminal sequencing	I	X		
	LC-MS/CE-MS	I/P	X	X	<736>
Association/aggregation	CE (free solution/gel filled)	I	X	X	<726> <1047>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
N-terminal blocking	N-terminal sequence	I	X		
	Peptide mapping				<1047>

Continued

Table 2. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
Phosphorylation	Colorimetry	I	X	X	
	Ion chromatography	I	X		
	Peptide mapping	I	X	X	<1047>
Molecular weight	SDS-PAGE	I/P	X	X	<1047>
	SDS-CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	HP-SEC	I	X	X	<621>
	LC-MS/CE-MS	I	X	X	<736>
Impurity profile	SDS-PAGE	I	X	X	<726>
					<1047>
	CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	SDS-CE	I	X	X	<1047>
	HP-IEC	I	X	X	<621>
	RP-HPLC	I	X	X	<621>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Spectrophotometry	I/P	X	X	
	NMR	I	X	X	
Residual DNA	Spectrophotometry/colorimetry	I	X	X	
	PCR	I	X	X	
Residual host-cell protein	Western blot	I	X	X	
Residual solvent/moisture	GC	I	X	X	<621>
	TGA	I/P	X	X	<891>
	NMR	I	X	X	
	Karl-Fischer	I/P	X	X	<541>
Assay (mass)	CE (free solution/gel filled)	I/P	X	X	<726>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HPLC (IEC, SEC, RP)	I/P	X	X	<621>
	Spectrophotometry/colorimetry	I/P	X	X	<851>
Ligand binding assay	Spectrophotometry/colorimetry	I/P	X	X	
	Fluorescence spectrophotometry	I/P	X	X	
	Fluorescence polarization measurements	I/P	X	X	
	SPR	I/P	X	X	
	Microarray (colorimetric, spectrophotometric, radionucleotide, or fluorescence detection)	I/P	X	X	
	Competitive inhibition	I/P	X	X	
	NMR (T_1 or T_2)	I	X		
	Equilibrium dialysis	I	X		
	HP-SEC—column saturation technique	I/P	X		
Secondary structure	CD	I	X		
	X-ray diffraction	I	X		
Solution conformation	CD	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		

Continued

Table 2. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
Crystal structure	X-ray crystallography	I/P	X		
Binding and Intercellular Transport	Electron microscopy	I	X		
	Video confocal microscopy	I	X		

*GC indicates *USP* General Chapter; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; CE, capillary electrophoresis; RP-HPLC, reversed phase-high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; SDS-CE, capillary electrophoresis in the presence of sodium dodecyl sulfate; LC-MS, liquid chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; HP-IEC, HPLC ion-exchange chromatography; NMR, nuclear magnetic resonance; CIEF, capillary isoelectric focusing; HP-SEC, HPLC size-exclusion chromatography; MALLS, multiple angle laser light scattering detection; PCR, polymerase chain reaction; TGA, thermogravimetric analysis; SPR, surface plasmon resonance; and CD, circular dichroism.

† I = Ingredient; P = Product.

‡ These tests are typically used as lot release tests; however, the actual lot release tests should be decided for individual intermediates (active ingredients) and products on a case-by-case basis based on characterization data, other pertinent information, and as needed to comply with regulatory requirements.

proteins at receptor sites. For example, human growth hormone acts by binding to 2 different receptor binding sites (I and II) to produce a biologic response. Small changes arising in a natural-source or rDNA nonglycosylated protein may occur and may be difficult to detect as in the following examples: (1) in the deamidation of an amino acid; (2) when the substitution of one amino acid is not available in sufficient amounts during synthesis; (3) during acetylation, as acetate levels in the fermentation process rise; (4) during oxidation; (5) in the event of incorrect incorporation of amino acids when mammalian codons are used in bacterial plasmids; (6) during improper posttranslational folding, which is affected by disulfide bonds; and (7) during carbamylation from process buffers such as urea, which may contain cyanate. Analytical procedures are increasingly powerful but still have limitations, including the following: (1) high-performance liquid chromatography (HPLC) may not detect an amino acid change "hidden" within the 3-dimensional structure of a protein; (2) mass spectrometry (MS) may require separation, affecting protein characteristics; (3) peptide maps may not always detect a change because of coelution; (4) pleiotropic proteins may require more than one bioassay (eg, growth hormone stimulates protein synthesis and lipolysis, inhibits insulin, stimulates new bone formation, and promotes erythropoiesis, with accelerated growth, reduction of adipose tissue, and increase in lean body mass); and (5) bioassays may not correlate well with human responses. For these reasons, analytical procedures alone, including bioassay, are limited in assessing the affect of change on the production of a nonglycosylated protein on clinical outcomes.

Glycosylated Proteins

Glycosylation is a posttranslation event that adds complex sugar (glycan) structures to specific amino acid sequences. Different glycan structures are added depending on the expression system used. For example, if a protein is expressed in *E coli,* no oligosaccharide moiety is added. If the protein is expressed in yeast, glycosylation will add only oligomannosyl oligosaccharide moieties. Addition of oligomannosyl oligosaccharide moieties is not observed in mammalian cell culture. If the protein is expressed in insect cells, glycosylation can add *N*-acetylglucosamine and fucose. If the protein is expressed in mammalian cells, glycosylation can add *N*-acetylneuraminic acid, and if the protein is expressed in plants, glycosylation can add xylose. Changes in glycosylation patterns can affect pharmacokinetics, pharmacodynamics, clinical outcomes, and stability; therefore analytical glycobiology is an important consideration when one assesses changes in the manufacturing process of natural-source and rDNA-derived glycosylated proteins. Because glycan composition and impurities vary with cell line, nutrients, purification process, and other factors, glycosylation pattern is also a useful process consistency marker during routine manufacturing. The glycan moiety or moieties of a glycoprotein can modify physical/chemical properties of a protein (eg, solubility, aggregation) and can create, modulate, or mask biologic binding and activity. With decreased glycosylation, binding to some receptors may be modulated. These effects can be manifested as changes in pharmacokinetics (eg, clearance), antigenicity, and activity. Despite its general importance, increased understanding of glycobiology indicates that glycosylation has varying degrees of effect. Glycosylation may directly affect activity, may indirectly affect activity (eg, through changes in pharmacokinetics), or may have no effect. Evaluation of this effect (and the effect of other posttranslational modifications) is thus an important part of any change control strategy. As with nonglycosylated proteins, full characterization of a glycosylated protein may not be possible, leading to a need for nonclinical and clinical studies to assess consistency in therapeutic outcomes in the presence of change.

Table 3. Physicochemical Tests for Nonglycosylated Protein Analysis*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
pH	pH	P	X	X	<791>
Isotonicity	Osmolality	P		X	
Identification of active ingredients	PAGE	I/P	X	X	<726> <1047>
	SDS-PAGE	I/P	X	X	<1047>
	Western blot	I/P	X	X	
	IEF	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727> <1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	CIEF	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	Peptide mapping	I/P	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X		
	Amino acid sequencing	I	X		
	Ligand binding activity (see later)	I/P	X	X	
	Enzyme activity (see later)	I/P	X	X	
Purity	PAGE	I/P	X	X	<726> <1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727> <1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	MALDI-TOF	I	X	X	<736>
	NMR	I	X	X	<761>
	Spectrophotometry/colorimetry	I/P		X	<851>
Dissociation/truncation/ deletion	SDS-PAGE	I	X	X	<1047>
	Western blot	I	X	X	
	SDS-CE (free solution/gel filled)	I	X	X	<1047>
	IEF	I	X		<1047>
	CIEF	I	X		<1047>
	MALDI-TOF/MS	I	X	X	<736>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X	X	
Oxidation	RP-HPLC	I/P	X	X	<621>
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X		
	C-terminal sequencing	I	X		

Continued

Table 3. *Continued*

Test	Procedure	I/P[†]	Characterization	Release[‡]	USP GC Link
	LC-MS/CE-MS	I/P	X	X	<736>
	CD	I	X		
Association/aggregation	PAGE	I	X	X	<726>
					<1047>
	CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
N-terminal blocking	N-terminal sequence	I	X		
	Peptide mapping				<1047>
Phosphorylation	Colorimetry	I	X	X	
	IEF	I			<1047>
	CIEF	I			<727>
					<1047>
	Ion chromatography	I	X		
	Peptide mapping	I	X	X	<1047>
Total sugar	Colorimetry	I	X		
Posttranslational modifications	IEF	I	X	X	<1047>
(other than those listed above)	CIEF	I	X	X	<1047>
	Peptide mapping	I	X	X	<1047>
	X-ray crystallography	I	X		
	CD	I	X	X	
Molecular weight	SDS-PAGE	I/P	X	X	<1047>
	SDS-CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	LC-MS/CE-MS	I	X	X	<736>
Impurity profile	PAGE	I	X	X	<726>
					<1047>
	SDS-PAGE	I	X	X	<1047>
	Western blot	I	X	X	
	CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	SDS-CE	I	X	X	<1047>
	HP-IEC	I	X	X	<621>
	RP-HPLC	I	X	X	<621>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Spectrophotometry	I/P	X	X	
	NMR	I	X	X	
Residual DNA	Spectrophotometry/colorimetry	I	X	X	
	PCR	I	X	X	
Residual host-cell protein	Western blot	I	X	X	

Continued

Table 3. *Continued*

Test	Procedure	I/P[†]	Characterization	Release[‡]	USP GC Link
Residual solvent/moisture	Karl-Fischer	I	X	X	<621>
	NMR	I	X	X	
Assay (mass)	PAGE	I/P	X	X	<726> <1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727> <1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HPLC (IEC, SEC, RP)	I/P	X	X	<621>
	Spectrophotometry/colorimetry	I/P	X	X	<851>
Assay activity (enzyme activity or inhibition)	Spectrophotometry/colorimetry	I/P	X	X	
	Fluorescence spectrophotometry	I/P	X	X	
	Fluorescence polarization measurements	I/P	X	X	
	SPR	I/P	X	X	
	Microarray (colorimetric, spectrophotometric, radionucleotide, or fluorescence detection)	I/P	X	X	
	Competitive inhibition	I/P	X	X	
	Noncompetitive inhibition				
	NMR (T_1 or T_2)	I	X		
	Equilibrium dialysis				
Ligand binding assay	Spectrophotometry/colorimetry	I/P	X	X	
	Fluorescence spectrophotometry	I/P	X	X	
	Fluorescence polarization measurements	I/P	X	X	
	SPR	I/P	X	X	
	Microarray (colorimetric, spectrophotometric, radionucleotide, or fluorescence detection)	I/P	X	X	
	Competitive inhibition	I/P	X	X	
	NMR (T_1 or T_2)	I	X		
	Equilibrium dialysis	I	X		
	HP-SEC—column saturation technique	I/P	X		
Denaturation	CD	I	X		
	X-ray crystallography	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		
Secondary structure	CD	I	X		
	X-ray diffraction	I	X		
Solution conformation	CD	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		
Crystal structure	X-ray crystallography	I/P	X		
Molecular shape/ hydrodynamic volume	Analytical ultracentrifugation	I	X		
Cell-surface or intercellular localization	Electron microscopy	I	X		
	Confocal microscopy	I	X		
Binding and intercellular transport	Electron microscopy	I	X		
	Video confocal microscopy	I	X		

Continued

Table 3. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
Molecular topography	Electron microscopy	I	X		
	Atomic force microscopy	I	X		

*GC indicates *USP* General Chapter; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing; CE, capillary electrophoresis; CIEF, capillary isoelectric focusing; HP-IEC, high-performance liquid chromatography (HPLC) ion-exchange chromatography; RP-HPLC, reversed-phase HPLC; HP-SEC, HPLC size-exclusion chromatography; LC-MS, liquid chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; NMR, nuclear magnetic resonance; MALLS, multiple angle laser light scattering detection; CD, circular dichroism; and SPR, surface plasmon resonance.

†I = Ingredient; P = Product.

‡These tests are typically used as lot release tests; however, the actual lot release tests should be decided for individual intermediates (active ingredients) and products on a case-by-case basis based on characterization data, other pertinent information, and as needed to comply with regulatory requirements.

Monoclonal Antibodies

Rapid advances in technology over the past several decades have resulted in increasing availability of therapeutic monoclonal antibodies, including abciximab (ReoPro), rituximab (Rituxan), daclizumab (Zenapax), basiliximab (Simulect), palivizumab (Synagis), infliximab (Remicade), trastuzumab (Herceptin), gemtuzumab ozogamicin (Mylotarg), alemtuzumab (Campath), adalimumab (Humira), and bevacizumab (Avastin). Immunoglobulin structure and function determinations have improved, with increased understanding of the complementarity-determining regions and the constant domains. Monoclonal antibodies can be grouped into 6 classes according to potential use: (1) binding to a cell surface target, with recruitment of immune response and target cell lysis; (2) binding to a cell surface receptor causing apoptosis; (3) cross-linking to a cell killing reagent; (4) binding to a target to block an interaction; (5) binding to a receptor to block a downstream process; and (6) catalysis. Analytical approaches for monoclonal antibodies include purity assays (ion-exchange chromatography, sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] or capillary electrophoresis, size-exclusion chromatography, isoelectric focusing [IEF] or capillary isoelectric focusing [CIEF]), identification assays (IEF or CIEF, capillary zone electrophoresis, peptide mapping), and biologic potency assays (binding assays or cell-based bioassays). Testing can occur either with or without digestion (eg, papain digestion) to assess the activity of each of the domains individually. Oligosaccharide residues on a monoclonal antibody add complexity and require consideration because they can be involved in activity. Immunoglobulin structure and function are inherently complex. Immunoglobulin G and immunoglobulin M antibodies interact via numerous pathways with the immune system. Changes in the manufacturing process can involve both physicochemical characterization studies as well as assessment of change in response in biological systems (bioassays). As with nonglycosylated and glycosylated proteins, full characterization of a monoclonal antibody may not be possible, leading to a need for nonclinical and clinical studies to assess therapeutic outcomes in the presence of change.

Procedures

Physicochemical Procedures

Natural-source and rDNA-derived peptides and proteins pose many analytical challenges arising from their complex structures, extent and nature of impurities (product, process, and contaminant impurities), pleiotropic biologic activities, and poor understanding of mechanism(s) of action. Typically, the numbers of assays that are performed for batch release are 3 to 4 times those used for a conventional small-molecule therapeutic. Impurities can include truncated forms, misincorporations, degradation products (eg, deamidated, oxidized, cleaved products), glycosylation errors, protein adducts, and host-cell contaminants (both proteins and DNA). Physicochemical measures may not correlate with enzyme-linked immunosorbent assays (ELISAs) or potency measurements because of variability and/or specificity issues. Many proteins can bind to multiple receptors, and in vitro/in vivo correlations can be difficult to establish. Analytical procedures are developed in association with the capability/limitations of the process, with method validation conducted to demonstrate what is "known" about protein structure and function. Analytical procedures offer an increasingly broad array of techniques that are used for identity, quantification, purity, structure, heterogeneity, activity, and stability studies. Despite a growing array of physicochemical information, variable effects in the clinic may still be observed in the presence of a manufacturing change. These effects sometimes occur in the absence of demonstrable physicochemical and biologic potency changes and sometimes do not occur even when significant changes in these parameters can be demonstrated. Development of characterization

Table 4. Physicochemical Tests for Glycosylated Protein Analysis*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
pH	pH	P	X	X	<791>
Isotonicity	Osmolality	P		X	
Identification of active ingredients	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	Western blot	I/P	X	X	
	IEF	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	CIEF	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	Peptide mapping	I/P	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X		
	Amino acid sequencing	I	X		
	Ligand binding activity (see later)	I/P	X	X	
	Enzyme activity (see later)	I/P	X	X	
Purity	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	MALDI-TOF	I	X	X	<736>
	NMR	I	X	X	<761>
	Spectrophotometry/colorimetry	I/P		X	<851>
Dissociation/truncation/ deletion	SDS-PAGE	I	X	X	<1047>
	Western blot	I	X	X	
	SDS-CE (free solution/gel filled)	I	X	X	<1047>
	IEF	I	X		<1047>
	CIEF	I	X		<1047>
	MALDI-TOF/MS	I	X	X	<736>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X	X	
Oxidation	RP-HPLC	I/P	X	X	<621>
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X		
	C-terminal sequencing	I	X		

Continued

Table 4. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
	LC-MS/CE-MS	I/P	X	X	<736>
	CD	I	X		
Association/aggregation	PAGE	I	X	X	<726>
					<1047>
	CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
N-terminal blocking	N-terminal sequence	I	X		
	Peptide mapping				<1047>
Phosphorylation	Colorimetry	I	X	X	
	IEF	I			<1047>
	CIEF	I			<727>
					<1047>
	Ion chromatography	I	X		
	Peptide mapping	I	X	X	<1047>
Total sugar	Colorimetry	I	X	X	
Monosaccharide composition analysis	HPAEC-PAD	I	X		
	RP-HPLC	I	X		
	CE (free solution)	I	X		
Oligosaccharide profile	HPAEC-PAD	I	X	X	
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
	MALDI-TOF	I	X	X	
	NMR	I	X		
Structures of oligosaccharides	HPAEC-PAD	I	X	X	
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
	MALDI-TOF	I	X	X	
	NMR	I	X	X	
Terminal galactose—exposed/unexposed	Enzyme digestion and colorimetry or HPAEC	I	X	X	
	Lectin binding assay/lectin affinity chromatography	I	X		
Presence of GalI1,3Gal	Lectin binding assay/lectin affinity chromatography with/without *l*-galactosidase digestion				
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
Glycomapping	Peptide mapping (with/without glycosidase digestion)	I	X	X	

Continued

Table 4. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
Posttranslational modifications (other than those listed above)	IEF	I	X	X	<1047>
	CIEF	I	X	X	<1047>
	Peptide mapping	I	X	X	<1047>
	X-ray crystallography	I	X		
	CD	I	X	X	
Molecular weight	SDS-PAGE	I/P	X	X	<1047>
	SDS-CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	LC-MS/CE-MS	I	X	X	<736>
Impurity profile	PAGE	I	X	X	<726>
					<1047>
	SDS-PAGE	I	X	X	<1047>
	Western blot	I	X	X	
	CE (free solution/gel filled)	I	X	X	<727>
					<1047>
	SDS-CE	I	X	X	<1047>
	HP-IEC	I	X	X	<621>
	RP-HPLC	I	X	X	<621>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Spectrophotometry	I/P	X	X	
	NMR	I	X	X	
Residual DNA	Spectrophotometry/colorimetry	I	X	X	
	PCR	I	X	X	
Residual host-cell protein	Western blot	I	X	X	
Residual solvent/moisture	Karl-Fischer	I	X	X	<621>
	NMR	I	X	X	
Assay (mass)	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HPLC (IEC, SEC, RP)	I/P	X	X	<621>
	Spectrophotometry/colorimetry	I/P	X	X	<851>
Assay activity (enzyme activity or inhibition)	Spectrophotometry/colorimetry	I/P	X	X	
	Fluorescence spectrophotometry	I/P	X	X	
	Fluorescence polarization measurements	I/P	X	X	
	SPR	I/P	X	X	
	Microarray (colorimetric, spectrophotometric, radionucleotide, or fluorescence detection)	I/P	X	X	

Continued

Table 4. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
	Competitive inhibition	I/P	X	X	
	Noncompetitive inhibition				
	NMR (T_1 or T_2)	I	X		
	Equilibrium dialysis				
Ligand binding assay	Spectrophotometry/colorimetry	I/P	X	X	
	Fluorescence spectrophotometry	I/P	X	X	
	Fluorescence polarization measurements	I/P	X	X	
	SPR	I/P	X	X	
	Microarray (colorimetric, spectrophotometric, radionucleotide, or fluorescence detection)	I/P	X	X	
	Competitive inhibition	I/P	X	X	
	NMR (T_1 or T_2)	I	X		
	Equilibrium dialysis	I	X		
	HP-SEC—column saturation technique	I/P	X		
Denaturation	CD	I	X		
	X-ray crystallography	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		
Secondary structure	CD	I	X		
	X-ray diffraction	I	X		
Solution conformation	CD	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		
Crystal structure	X-ray crystallography	I/P	X		
Molecular shape/ hydrodynamic volume	Analytical ultracentrifugation	I	X		
Cell-surface or intercellular localization	Electron microscopy	I	X		
	Confocal microscopy	I	X		
Binding and intercellular transport	Electron microscopy	I	X		
	Video confocal microscopy	I	X		
Molecular topography	Electron microscopy	I	X		
	Atomic force microscopy	I	X		

*GC indicates *USP* General Chapter; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing; CE, capillary electrophoresis; CIEF, capillary isoelectric focusing; HP-IEC, high-performance liquid chromatography (HPLC) ion-exchange chromatography; RP-HPLC, reversed-phase HPLC; LC-MS, liquid chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; NMR, nuclear magnetic resonance; HP-SEC, HPLC size-exclusion chromatography; MALLS, multiple angle laser light scattering detection; HPAEC-PAD, high pH anion-exchange chromatography with pulsed amperometric detection; PCR, polymerase chain reaction; SPR, surface plasmon resonance; and CD, circular dichroism.

†I = Ingredient; P = Product.

‡These tests are typically used as lot release tests; however, the actual lot release tests should be decided for individual intermediates (active ingredients) and products on a case-by-case basis based on characterization data, other pertinent information, and as needed to comply with regulatory requirements.

Table 5. Physicochemical Tests for Monoclonal Antibodies Analysis*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
pH	pH	P	X	X	<791>
Isotonicity	Osmolality	P		X	
Identification of active ingredients	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	Western blot	I/P	X	X	
	IEF	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	CIEF	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	Peptide mapping	I/P	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X		
	Amino acid sequencing	I	X		
	Binding Activity (see later)	I/P	X	X	
Purity	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HP-IEC	I/P	X	X	<621>
	RP-HPLC	I/P	X	X	<621>
	LC-MS/CE-MS	I/P	X	X	<736>
	MALDI-TOF	I	X	X	<736>
Dissociation/deletion	SDS-PAGE	I	X	X	<1047>
	Western blot	I	X	X	
	SDS-CE (free solution/gel filled)	I	X	X	<1047>
	IEF	I	X		<1047>
	CIEF	I	X		<1047>
	MALDI-TOF/MS	I	X	X	<736>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	Peptide mapping	I	X	X	<1047>
	N-terminal sequencing	I	X	X	
	C-terminal sequencing	I	X	X	
Oxidation	RP-HPLC	I/P	X	X	<621>
	Peptide mapping	I	X	X	<1047>
	LC-MS/CE-MS	I/P	X	X	<736>
	CD	I	X		
Association/aggregation	PAGE	I	X	X	<726>
					<1047>

Continued

Table 5. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
	CE (free solution/gel filled)	I	X	X	<727> <1047>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
N-terminal repeat sequence	N-terminal sequence	I	X		
	Peptide mapping				<1047>
Total sugar	Colorimetry	I	X	X	
Monosaccharide composition analysis	HPAEC-PAD	I	X		
	RP-HPLC	I	X		
	CE (free solution)	I	X		
Oligosaccharide profile	HPAEC-PAD	I	X	X	
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
	MALDI-TOF	I	X	X	
	NMR	I	X		
Structures of oligosaccharides	HPAEC-PAD	I	X	X	
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
	MALDI-TOF	I	X	X	
	NMR	I	X	X	
Terminal galactose—exposed/unexposed	Enzyme digestion and colorimetry or HPAEC	I	X	X	
	Lectin binding assay/lectin affinity chromatography	I	X		
Presence of GalI1,3Gal	Lectin binding assay/lectin affinity chromatography with/without *l*-galactosidase digestion				
	RP-HPLC with fluorescent detection	I	X	X	
	CE (free solution)	I	X	X	
	LC-MS/CE-MS	I	X	X	
Glycomapping	Peptide mapping (with/without glycosidase digestion)	I	X	X	
Molecular weight	SDS-PAGE	I/P	X	X	<1047>
	SDS-CE (free solution/gel filled)	I	X	X	<727> <1047>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Analytical ultracentrifugation	I	X		
	LC-MS/CE-MS	I	X	X	<736>
Impurity profile	PAGE	I	X	X	<726> <1047>
	SDS-PAGE	I	X	X	<1047>
	Western Blot	I	X	X	
	CE (free solution/gel filled)	I	X	X	<727> <1047>

Continued

Table 5. *Continued*

Test	Procedure	I/P†	Characterization	Release‡	USP GC Link
	SDS-CE	I	X	X	<1047>
	HP-IEC	I	X	X	<621>
	RP-HPLC	I	X	X	<621>
	LC-MS/CE-MS	I	X	X	<736>
	HP-SEC	I	X	X	<621>
	HP-SEC/MALLS	I	X	X	
	Spectrophotometry	I/P	X	X	
	NMR	I	X	X	
Residual DNA	Spectrophotometry/colorimetry	I	X	X	
	PCR	I	X	X	
Residual host-cell protein	Western blot	I	X	X	
Assay (mass)	PAGE	I/P	X	X	<726>
					<1047>
	SDS-PAGE	I/P	X	X	<1047>
	CE (free solution/gel filled)	I/P	X	X	<727>
					<1047>
	SDS-CE (free solution/gel filled)	I/P	X	X	<1047>
	HPLC (IEC, SEC, RP)	I/P	X	X	<621>
	Spectrophotometry/colorimetry	I/P	X	X	<851>
Ligand binding assay	Fluorescence spectrophotometry	I/P	X	X	
	SPR	I/P	X	X	
Denaturation	CD	I	X		
	Fluorescence spectrophotometry	I	X		
	NMR (T_1 or T_2)	I	X		
Secondary structure	CD	I	X		

*GC indicates *USP* General Chapter; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; IEF, isoelectric focusing; CE, capillary electrophoresis; CIEF, capillary isoelectric focusing; HP-IEC, high-performance liquid chromatography (HPLC) ion-exchange chromatography; RP-HPLC, reversed-phase HPLC; LC-MS, liquid chromatography-mass spectrometry; CE-MS, capillary electrophoresis-mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; NMR, nuclear magnetic resonance; HP-SEC, HPLC size-exclusion chromatography; MALLS, multiple angle laser light scattering detection; HPAEC-PAD, high pH anion-exchange chromatography with pulsed amperometric detection; PCR, polymerase chain reaction; SPR, surface plasmon resonance; and CD, circular dichroism.

†I = Ingredient; P = Product

‡These tests are typically used as lot release tests; however, the actual lot release tests should be decided for individual intermediates (active ingredients) and products on a case-by-case basis based on characterization data, other pertinent information, and as needed to comply with regulatory requirements.

data versus mechanism of action may be used to develop a risk strategy in the presence of change for protein-based therapeutics, ranging from very low risk to high risk situations.

Biological Assays

A biologic assay is defined as an analytical procedure measuring a biological activity of a test substance based on a specific, functional, biological response of a test system.[22,23]

Bioassays can rely on animals, in vitro cell lines, cell-based "biochemical" assays (kinase receptor activity, reporter genes), binding assays (immunoassays, biosensors), and enzyme assays. Bioassays are the only nonclinical tests that indicate a product is biologically active. They are informative in equivalence studies only to the extent that a change affects a part of the protein that affects activity. They can be limited in assessing the effect on some parameters (eg, pharmacokinetics) but critical in assessing immunogenicity. An immune response is assessed through measurement of antibody production. Only bioassays can confirm if these antibodies neutralize biologic effect. Bioassays also are critical for structure/function studies. Although physicochemical procedures can detect the majority of modifications that occur with change, the effect of these changes can be assessed only when the physicochemical change is correlated with biological activity. The

most well-characterized, precise bioassay is generally selected as the one for lot release potency. Care must be taken in this selection because of the pleiotropic activities of many proteins. When the mechanism of action is unknown or complex (eg, therapeutic vaccines), bioassays may be limited in value. Bioassays also are limited by a high degree of variability, which is generally higher for animal models, lower for cell-based bioassays or biochemical bioassays, and best with ligand binding/enzymes. Bioassays may not be needed for all peptide therapeutics but are of value for nonglycosylated and glycosylated proteins, particularly if they are stability indicating. They are also useful screening tools to assess relevance of changes in complex glycosylation patterns, particularly if they are in vivo based. Bioassays are also critical determinants of potency at the time of lot release and thus are of value even when no change occurs. The end result of this type of testing is a relative potency measure, expressed as units (or international units [IU])/mass of product. Potency is measured against international, USP, or in-house standards, or a predicate batch. This type of testing assesses batch-to-batch consistency against an equivalence interval of 100% of labeled claim. Bioassay testing for consistency, comparability, and equivalence relies on a determination of parallelism. Recent work at USP has suggested that improvements are needed in the statistical assessment of parallelism.[24]

Pharmacological and Toxicological Procedures

Experience during the past 2 decades has shown that the consequences of change in the manufacture of a natural-source or rDNA protein are not always predictable using nonclinical studies. A key question thus arises: What effect will one or more changes that occur during the course of product development have on the product's safety and biologic activity? The significance of a change can at times be assessed using assay/model systems that have been shown to be sensitive to a change. The types and timing of changes and the knowledge gained from past experience are thus inextricably linked to the design of pharmacology/toxicology studies to support an equivalence assessment. Current challenges in assessing equivalence of proteins include assay sensitivity and availability, lack of standards (positive and negative controls, reference standards), product availability from earlier processes for optimization of bridging studies, complications related to host-cell and process-related impurities, and limitations of animal models in predicting human effects due to species specificity.

Improved predictive value of preclinical safety studies has benefited from the ICH approaches and continues to improve with validation and acceptance of alternative methods, use of nontraditional animal models, technological advances, increasing reliance on surrogate and biomarkers, and other approaches. Comparison of conventional (small-molecule) therapeutics with natural-source and rDNA-derived protein therapeutics reveals both differences and similarities, some of which add additional study burden for second-entry interchangeable protein therapeutics, where issues of equivalence are involved. For example, many biological products are simple solution formulations given by injection that obviate the need to show bioequivalence. Because no drug or biological is 100% safe, the management of risks becomes a crucial factor in demonstrating equivalence. The use of appropriate animal models during development and manufacture of these products may provide supportive data for an equivalence determination, as it does now for conventional pharmaceuticals.

Pharmacokinetics

Pharmacokinetic studies are highly useful in assessing the impact of a change in the manufacture of a natural-source or rDNA-derived protein. An important feature of these studies is variability in absorption, distribution, and elimination. The sources of variability that can affect equivalence are intrinsic (physical and chemical properties, structural, genetic) or extrinsic (physiology, demographics, disease conditions). Although most proteins are administered by injection, absorption can vary depending on whether administration is subcutaneous, intramuscular, or intravenous. Formulation differences have also been shown to affect pharmacokinetics, even if the same route of administration is used. A variety of factors, including receptor density, physiological factors, glycosylation state, and physicochemical characteristics can affect distribution. Both oxidation and glycosylation are known to affect pharmacokinetics and, in certain settings, pharmacodynamics. Many investigations in both human and nonhuman species have shown that elimination is affected by protein molecular weight; examples exhibit many-fold differences in half-life. For proteins that are rapidly cleared by the liver, hepatic blood flow (which increases during exercise) can influence both the pharmacokinetics and pharmacologic effects. For these reasons, pharmacokinetics reflective of tissue/organ distribution as well as systemic exposure measures, if relevant, may be useful in assessing equivalence. Pharmacokinetic studies for equivalence determinations on a solution of natural-source or rDNA-derived protein can be used to confirm the identity of the active ingredient (ie, they are useful in establishing pharmaceutical equivalence). Design of a study will depend on the protein therapeutic, taking into account half-life, endogenous levels, need to study healthy versus patient volunteers, ethical considerations, and other factors. Standard approaches to equivalence now used in bioequivalence studies can be used to make comparisons.

Pharmacodynamics

Pharmacodynamic studies may be even more useful than pharmacokinetic studies, given that they more directly reflect clinical outcomes and can change even when pharmacokinetic measures do not. Despite these advantages, pharmacodynamic studies also pose many challenges, including choice of study population, high inter- and intra-subject variability, change with disease progression, and difficulty in interpretation because of pleiotropism, product-related substances, and process- and product-related impurities. An example is intravenous immunoglobulin, which exhibits multiple mechanisms of action, multiple components in a preparation, and a high degree of inter- and intra-subject variability in clinical outcomes. Pharmacodynamic studies may not always allow focus on the ultimate clinical benefit because of disease progression, study duration, ethical issues, and other factors. Instead, pharmacodynamic studies usually focus on a surrogate or biomarker of interest that waxes and wanes over a time period that allows adequate study. Pharmacodynamic studies allow comparisons between pre- and postchange dosage forms, by facilitating comparison of a suitable surrogate or biomarker (eg, platelet aggregation following administration of antiplatelet therapy in the treatment of myocardial infarction). Direct comparisons rely on measures similar to those for pharmacokinetic studies (eg, area under the effect curve/AUEC and peak effect/Cpeak). More complex pharmacokinetic and pharmacodynamic modeling may also provide better mechanistic understanding, verify kinetic equivalence, and allow discrimination of "system" versus product variability. The benefit may also be limited because of high variability, low precision and accuracy, and ethical difficulty in approaching maximal effect. Because maximal effect is related to receptor number, it can reflect changes more of the in vivo system itself rather than the protein under investigation. Because the focus of a pharmacodynamic study is on a specific end point related to the natural-source or rDNA biologic, a case-by-case approach is generally needed.

Clinical Efficacy and Safety

Clinical studies may be used to assess equivalence using both safety and efficacy end points. They are used in this context now to show equivalence for some conventional pharmaceuticals where reliance on systemic exposure measures is not suitable. Noninferiority studies have been considered in detail in the ICH E10 guidance.[25] According to the E10 approach, a new drug or regimen may have benefits with respect to a primary or secondary end point in comparison to the existing drug or regimen. In such cases, it is not necessary for the new regimen to be superior to the existing regimen with respect to all the end points. For example, if survival is the primary end point, the new regimen with an improved safety profile need only be similar with respect to survival in order to be the preferred regimen. The noninferiority term captures the one-sidedness of the primary hypothesis (eg, the product after a change can be better for some end points but should remain within a specified lower bound margin for others). The approach is applicable to equivalence testing for a natural-source or rDNA-derived protein, where both noninferiority and nonsuperiority would be assessed. With this approach, relevant clinical outcomes should stay within both upper and lower bounds, which is the equivalence interval.

Immunogenicity

Serious adverse events (eg, pure red cell aplasia) have raised concerns about postapproval change both within and between manufacturers of natural-source and rDNA-derived proteins. Despite these high-profile examples, many exogenously administered proteins produce an antibody response with little or no clinical consequence. A review of available data based on several decades of experience with rDNA-derived proteins will be highly useful. Because important immune responses occur infrequently in a population, prospective, randomized clinical trials may be of limited value. In this context, some type of market surveillance may be needed. Many factors affect immunogenicity of protein therapeutics, including structural alterations (aggregation, oxidation, deamidation and degradation, and conformational changes), storage conditions, production/purification techniques, formulation, route of administration, dose and frequency of administration, immunity status, and genotype of patient. Immunogenicity may have no clinical effect (insufficient antibody production, minimal or transient patient response) or may produce a spectrum of responses (hypersensitivity, change in protein pharmacokinetics, neutralization of biologic effect[s] of the therapeutic protein, neutralization of biologic effect[s] of a family of protein therapeutics, and/or neutralization of endogenous protein). Antibodies may accelerate or retard therapeutic protein clearance. Antibodies can be detected using a variety of approaches. Each should be sensitive, specific, and able to detect low-affinity antibodies. It is important to fully characterize an immune response using both immunoassays, which detect antibodies that bind to the drug, and biologic assays, which detect neutralizing antibodies that block biologic effects. Platforms for antibody detection include radioimmunoassay, ELISA, and surface plasmon resonance. Biological assay platforms use a variety of end points (see above) and either primary cells or engineered cell lines. A human immune response cannot be predicted based on animal testing.

The Role of the United States Pharmacopeia

Monographs

A public monograph in *USP–NF* helps practitioners and other interested parties understand how an article's strength, quality, and purity should be controlled. Various terms are used to express the overall quality of a therapeutic article—ICH: quality; FFDCA: identity, strength, quality, purity, and potency; and PHSA: safety, purity, and potency. By the use of appropriate naming conventions, they support "clear, useful" names that help practitioners intelligently and safely use a therapeutic product. The case for a public monograph to support equivalence testing can also be made. WHO and various regulatory agencies subdivide equivalence approaches into (1) pharmaceutical equivalence (same active ingredient, same dosage form, same route of administration, and the same strength or concentration), and (2) bioequivalence (same rate and extent of availability after administration at the same concentration). For solid dosage forms containing noncomplex (pharmaceutical) drug substances, equivalence experiments focus on bioequivalence, given that documentation of pharmaceutical equivalence for a well-characterized active ingredient is relatively easy. In this context, a modern USP monograph is at least a start—and sometimes more than a start—in determining pharmaceutical equivalence. For a dosage form containing a biological or biotechnological drug substance, the emphasis is on pharmaceutical equivalence, because dosage forms of these substances are mostly parenteral solutions. Bioequivalence, which focuses on comparative release of the drug substance from test and reference dosage forms, is considered self-evident for parenteral solutions. Pharmaceutical equivalence experiments of 2 dosage forms containing a noncomplex active ingredient focus on the active ingredient itself, given that the remaining elements of pharmaceutical equivalence are generally satisfied without debate. At 21 *Code of Federal Regulations* (*CFR*) 320.22(b)(1)(ii) excipients for a parenteral dosage form submitted pursuant to 505(j) must be qualitatively and quantitatively identical to the reference listed drug. This may not be possible for interchangeable generics containing complex active ingredients. Again, for a complex active ingredient, a *USP* monograph is a start, and sometimes more than a start, in determining pharmaceutical equivalence for natural-source or recombinant complex active ingredients. It creates a foundation to which all manufactured ingredient and product articles should conform. Despite the value of a general public standard, the monographs for a biological and/or biotechnological drug substance and dosage form should be flexible to account for different impurities, especially when the manufacturing processes are different. This also is true of noncomplex active ingredients and products. Unlike other pharmacopeias, *USP* generally does not provide process information in a monograph, except to the extent that it defines an article as synthetic, natural, or biotechnological. Thus, a *USP* monograph is a starting point for subsequent-entry manufacturers, recognizing that substantial additional one-time characterization studies may be needed to document equivalence. Review of these studies is the province of the regulatory agency.

At this time, public monographs in *USP* are available for only a few rDNA-derived protein-based therapeutics. They also are not available in *CFR*. This differs from the approach used for antibiotics, which historically parallels in some ways the evolution of control for rDNA-based therapeutics. In the case of antibiotics, the United States initially required extensive governmental lot release testing without reliance on USP monographs and official USP Reference Standards. To satisfy the need for public monographs, the government published antibiotic monographs in *CFR*. With advances in analytical procedures and manufacturing capability, the United States government abandoned antibiotic lot release testing and, with deletion of Section 507 of FFDCA in the 1997 FDAMA, terminated *CFR* publication of public antibiotic monographs. Since then USP has developed full monographs for antibiotics, working collaboratively with FDA. USP has worked collaboratively with FDA on antibiotic reference standards since the 1970s, when the FDA antibiotic reference standard program was transferred to USP. In contrast, FDA has not promulgated public monographs for natural-source or rDNA-derived proteins, either in *CFR* or by working collaboratively with USP. There are also few public standards for these articles and little or no public collaborative testing of them.

General Chapters

In assessing equivalence between 2 complex actives-based dosage forms, a key question is how much additional characterization data are needed beyond the tests in a *USP–NF* monograph. USP is working to make its monographs more complete and flexible in order to account for different routes of synthesis and different impurity profiles. Nonetheless, the additional studies needed to confirm equivalence for 2 biologicals drawn from different sources may require comparative clinical, pharmacodynamic, pharmacokinetic, and other nonclinical studies. These one-time characterization studies are beyond the scope both of a private standard and standards in a public pharmacopeial monograph. Nonetheless, in General Chapters, USP can create useful techniques that form the basis for private characterization studies that support both public and private standards. To the extent that these can be prospectively harmonized they are even more valuable. Maps of *USP–NF* General Chapters useful to

manufacturers of protein-based therapeutics are shown in Figures 2 and 3.

Reference Standards

Official USP Reference Standards, generally referenced in monographs or in General Chapters, are highly purified physical materials that are approved by the USP Reference Standards Committee. There are 6 different types of reference standards, and each can play an important role in equivalence studies.

Drug Substance Reference Standards

These are the traditional USP reference standards, used in important tests of a monograph. In general, they are articles of commerce donated to USP. In USP laboratories, they undergo careful recharacterization testing and collaborative studies to

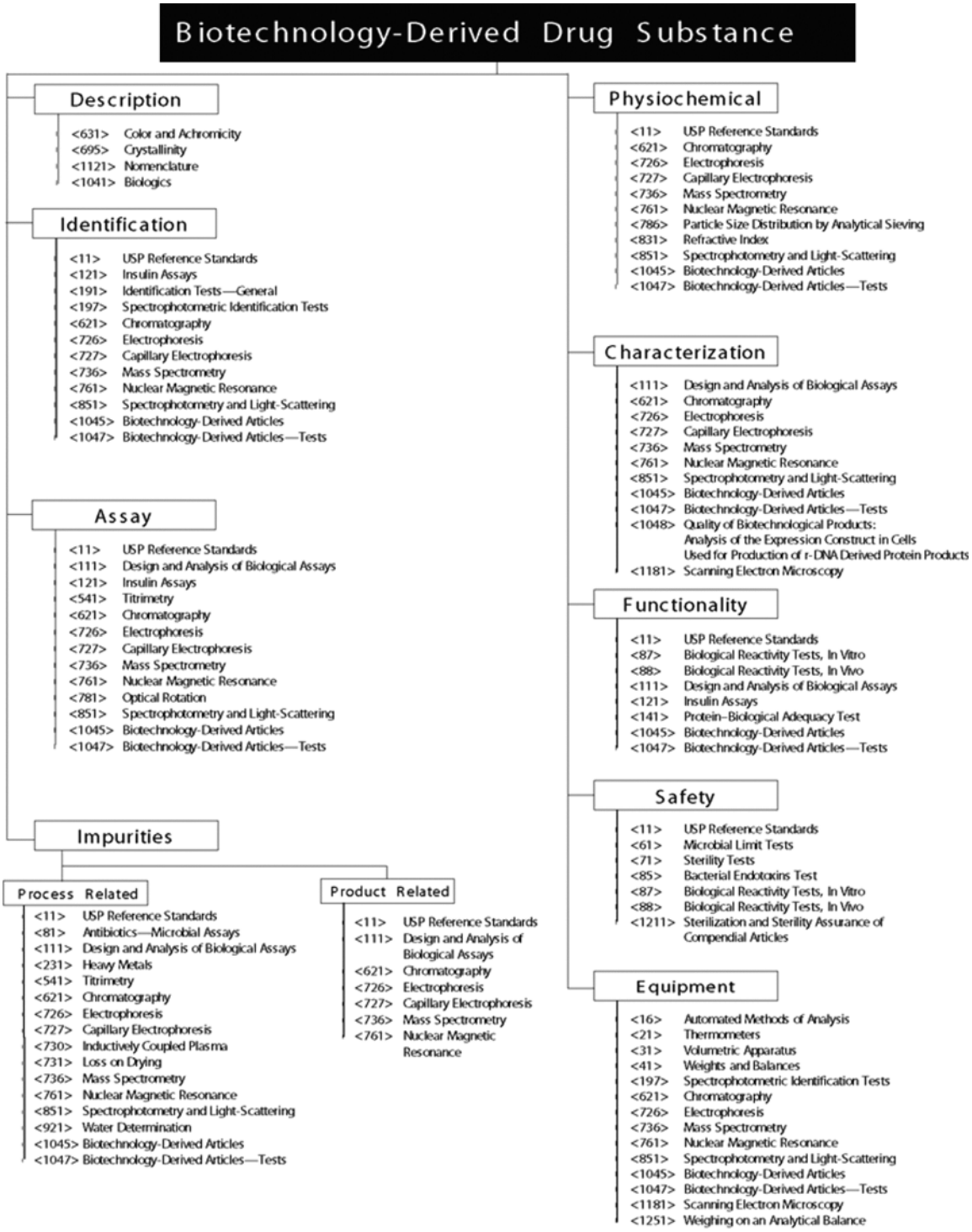

Figure 2. Biotechnology-derived drug substances. (Reproduced with permission from USP.)

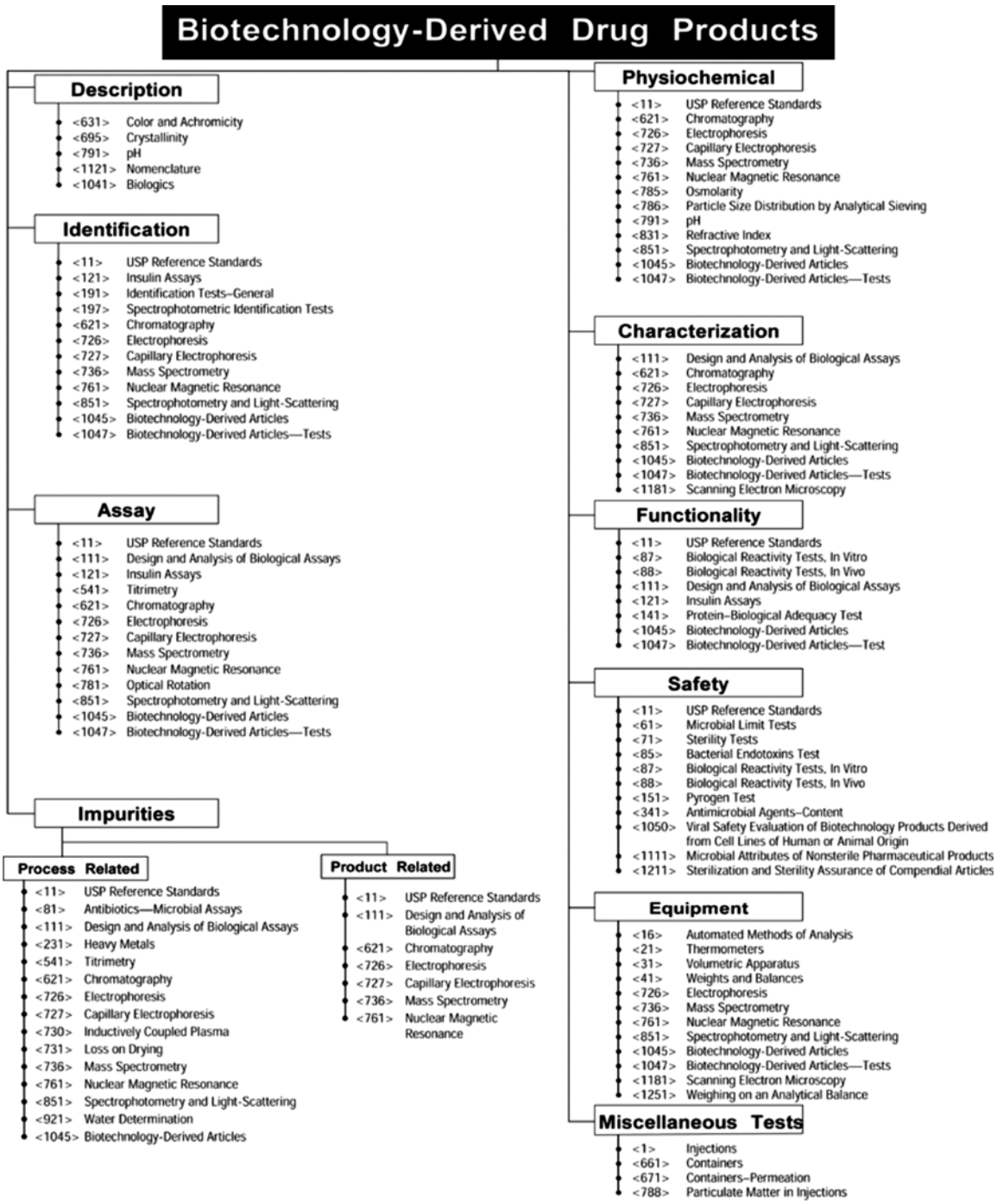

Figure 3. Biotechnology-derived drug products. (Reproduced with permission from USP.)

assess content. By comparison with noncomplex products, collaborative studies for natural-source and rDNA-derived protein therapeutics involve more laboratories because of the variability of the assays, especially bioassays in animals or cell-based tests. Potency in USP units is assigned to the reference standard based on these collaborative studies.

Drug Product Reference Standards

In general, a drug substance reference standard is also used for procedures that assess the drug product. By its very nature, the manufacture of complex active products sometimes bypasses the drug substance stage and goes directly to either a concentrate or a finished product. Because equivalent products do not always have to have identical excipients, the presence of different excipients in each of the products may interfere with the tests and assays. This issue requires resolution by a manufacturer working with USP to ensure compliance with the compendial monograph.

Impurities Reference Standards

Equivalence studies involving 2 complex products that are produced using different routes (eg, yeasts, *E coli*, animal,

or human cells) should take into consideration the impurity profiles of each of the resulting products. Because of the different vectors, the final impurity profiles will have, in all likelihood, similar or different impurities at different levels. Because impurity profiles are a factor in the safety and efficacy of products, their determination and quantitation will require the use of more than one impurity reference standard.

Procedural Reference Standards

This reference standard may be used by analysts during procedure development and validation, as well as in routine tests to ensure that the procedure, under the conditions of use, is working as intended and as validated. It is generally recognized that bioassays are highly variable. In equivalence studies, especially for complex active substances and products, the relative lack of accuracy and precision can bias an equivalence determination. A procedural reference standard will standardize the procedure used regardless of the product, thus reducing the uncertainty of the results. Procedural reference standards are being developed for methods used to characterize complex actives products (eg, amino acid analysis).

Ancillary Materials Reference Standards

Ancillary materials are chemical or biological substances used during the manufacture of complex actives products and are not intended to remain in the final product. The quality and the performance of these materials are part of the overall quality requirements of the finished product in order to ensure consistency among batches of final products. Furthermore, residues of these ancillary materials should not be present in the final products. Standardization of the ancillary materials requires the use of reference standards for comparison purposes. Equivalence studies of products with different ancillary material profiles will require testing of the final products for different residuals, depending on the manufacturing process.

Reagents Reference Standards

The reliability of noncompendial and compendial tests and assays—once the drug substance reference standards, the procedures used via procedural reference standards, and the ancillary materials have been standardized—depends on the quality of the reagents used in these assays and tests. Variability in tests and assays can be introduced by variability of reagents, which of course will bias the results as attempts are made to determine the equivalence of products. USP is developing reagents reference standards for complex actives.

SUMMARY

This report summarizes USP perspectives on equivalence approaches for complex active ingredients and dosage forms. The report focuses on protein-based therapeutics, with the understanding that approaches and principles for these articles may be generally applicable to other biological and biotechnological products. The primary responsibility for documenting equivalence rests with pharmaceutical manufacturers. Given various safety and other considerations, these data will usually require regulatory review. In this regard, regulatory agencies can assist manufacturers by delineating in regulation or guidance the type and amount of data needed depending on the type of change. Depending on the article and its safety and efficacy profiles, this information will need to be determined case by case. Public or private prior knowledge will assist manufacturers in making changes—without reliance on this prior knowledge, a full complement of safety and efficacy studies would be needed to justify even minor changes. Regulatory judgments based on prior experience are critical, given that requirements can be made so stringent that no manufacturing change would be allowed. The argument that manufacturers should develop optimal information about their processes and how they influence the strength, quality, and purity and also safety and efficacy of a manufactured article applies to all manufacturers.[26] It is not sufficient, however, to say that the required information to support a change can reside only with one manufacturer. Rather, any manufacturer of a natural-source or rDNA-derived protein therapeutic should conduct the characterization studies to support the necessary specifications for ingredients and final products.[27,28] The specifications may be used to allow batch release and assess batch-to-batch consistency. The information to document comparability and/or equivalence in the presence of change is a separate set of information, as discussed in this article. USP can provide public monographs that provide a baseline set of quality requirements for all manufacturers. In addition, USP can provide official USP Reference Standards for articles, impurities, procedures, ancillary materials, and reagents. Taken together, the various manufacturer, regulatory, and compendial risk-based approaches can assure the public that complex active ingredients and their dosage forms will be safe, effective, and equivalent from batch to batch and in the presence of intra- and intermanufacturer changes in components and composition and method of manufacture.

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APPENDIX I: USP HISTORY

1985 USP Convention adopts a resolution to explore the "feasibility and advisability of developing compendia monographs for macromolecular drugs derived from biotechnological processes."

1987 Initiation of a biotechnology program at USP.

1988 Appointment of a "Biotechnology Core Group" of the USP Subcommittee on Biochemistry and Microbiology.

Biotechnology Core Group issues statement in the November-December 1988 *Pharmacopeial Forum:* "Development of Compendial Monographs for Macromolecular Drugs and Devices Derived from Biotechnological Processes."

1988 Appointment of an Expert Advisory Panel on Biotechnology.

1989 *Stimuli to the Revision Process* article published in the July-August *Pharmacopeial Forum*, "Issues and Concepts Regarding Compendial Requirements for Biotechnology Products" by Pharmaceutical Manufacturers Association Biotechnology Committee, Quality Control Section.

1989 USP convenes the "USP Open Conference on Biotechnology-Derived Products," September 25-27 in Corpus Christi, Texas. Proceedings published in 1990.

1990 Eight biotechnology-derived products published in *Pharmacopeial Forum* under *Pharmacopeial Previews:*

- Alteplase and Alteplase for Injection
- Interferon alfa-2b and Interferon alfa-2b for Injection
- Somatrem and Somatrem for Injection
- Somatropin and Somatropin for Injection

Also published: A General Chapter on "Electrofocusing."

1992 "USP Rationale for the Development of Public Standards for Biological Products Licensed by FDA Center for Biologics Evaluation and Review" published as a *Stimuli for the Revision Process* article in *Pharmacopeial Forum*.

General Information Chapter <1045> *Biotechnology-Derived Articles* published in USP XXII, *7th Supplement*.

1995 Formation of a USP Subcommittee on Biotechnology and Biopolymers.

1995 "Development of Public Standards for Vaccines, Blood, and Allied Products—A Statement of Objectives" by the USP Microbiology Subcommittee published in the July-August *Pharmacopeial Forum*.

1998 Appointment of an Advisory Panel on Cell and Gene Therapy.

2000 Formation of USP Expert Committees on the following:

- Biotechnology and Natural Therapeutics and Diagnostics;
- Cell and Gene Therapy and Tissue Engineering;
- Blood and Blood Products;
- Vaccines, Virology, and Immunology.

Formation of the Complex Actives Division in the Information and Standards Development Department of USP.

2000 USP/FDA/International Association for Biological Standardization jointly sponsored Open Conference on Biologics.

2002 General Information Chapter <1047> *Biotechnology-Derived Products—Tests* published in *USP 25–NF 20*.

General Information Chapter <1046> *Cell and Gene Therapy Products* published in the *First Supplement* to *USP 25–NF 20*.

2003 USP Conference on Biological and Biotechnological Drug Substances and Products, Arlington, Virginia.

2004 Proposal in *PF* for Chapter <1403> *Ancillary Materials for Gene and Cell Therapy*.

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